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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES**

Appellants: PADGETT, et al Atty. Dkt. P-LG 4878
Serial No.: 10/066,390
Filed: FEBRUARY 1, 2002
Examiner: Jeffrey M. FREDMAN Art Unit: 1637
For: A METHOD OF INCREASING COMPLEMENTARITY
IN A HETERODUPLEX

BRIEF ON APPEAL

Mail Stop Appeal Brief-Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

June 29, 2006

Sir:

The present appeal is taken from the action of the Examiner in finally rejecting this application. Claims 66-72, 78-83, 85 and 87-90, which were pending of the time of the final rejection. These claims were not amended after the final action. A clean copy of these claims, double-spaced, appears in the appendix to this brief.

This brief was due May 29, 2006. A petition for a one-month Extension of Time is enclosed making this brief timely filed. See 37CFR1.117(a).

Please charge the fee for filing a brief in support of an appeal of \$ 250.00 to the Deposit Account No. 500933, and please credit any excess fees to such Deposit Account. A duplicate of this sheet is attached.

I hereby certify that three copies of this Appeal Brief with an Appendix (23 pages) is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service, on the date indicated above and is addressed to the Mail Stop Appeal Brief - Patents, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313 - 1450.

By: John E. Tarcza (John E. Tarcza)

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Brief on Appeal

(1) Real Party of Interest

The real party of interest is Large Scale Biology Corporation, a California Corporation having its principle offices at 3333 Vaca Valley Parkway, Suite 900, Vacaville, CA 95688. The inventors have assigned their rights in an assignment recorded March 19, 2002 on reel 013020, frame 0150.

(2) Related Appeals and Interferences

There are no related appeals or interferences. Several related pending applications have been filed but none are on appeal or in interference.

(3) Status of Claims

Claims 66-72, 78-83, 85 and 87-90 are pending, all of which were rejected and are on appeal. Claims 1-65, 73-77, 84 and 86 have been canceled without prejudice. A copy of the claims on appeal is attached hereto.

(4) Status of Amendments

No amendments after final rejection were submitted. The most recent amendment of November 15, 2005 was considered.

(5) Summary of Invention

Historically, many approaches have been tried to develop “new and improved” versions of a gene. All of these have succeeded commercially. Initially, scientists looked for additional versions of the same gene in different individuals, different strains or in different species. Later scientists exposed the gene or its host to various mutagens to randomly alter the gene in hope of producing a “new and improved” version. More recently, scientists have used a site-specific mutagenesis technique that converts one or more nucleotides in a polynucleotide specifically. (e.g., Estell et al., U.S. Patent 4,760,025) Even more recently scientists have started with two versions of the same gene, cleaved each into many pieces with sticky ends, allowed the sticky ends to anneal randomly by denaturing and renaturing where each fragment acts as a primer, and extending the primer to form a larger shuffled polynucleotide. (e.g., Stemmer, U.S. Patent 6,117,679). Only this last method produces sizable populations of sequence variants, but because the polynucleotides are fragmented and shorter fragments tend to preferentially be amplified, this technique tends to produce truncated versions of the gene being shuffled.

The presently claimed invention relates to methods for preparing many modified versions of a gene. The modifications occur in the nucleotide sequence. The modified versions or sequence variants result in new genes with different properties or which encode for different proteins with different properties.

The basic technology involves shuffling differences between two (or more) different genes. For example when two versions of the same gene (such as a human and mouse version) are compared, most of the sequence is the same in each parent gene with several point differences. The goal of the present invention is to reassort or shuffle these differences between the two to create a new molecule (or a population of new molecules) with some differences from the first parent and other differences from the second parent.

A given new molecule from such a population may result in biological activity intermediate between the parent molecules or it may have no biological activity at all or greater or less biological activity. Depending on the gene in question, it may be desirable to have any of these activities. Furthermore as many genes and their encoded proteins have multiple

activities, it may be desirable to have a molecule with greater activity for one property and lesser activity for another property.

The claimed method is illustrated by Figure 1 showing a very simple model system. One begins with at least two different parent polynucleotides. In the Figure, they have two polymorphisms, one at each site. The parent double stranded polynucleotide molecules are denatured into single strands and then allowed to hybridize. While some strands hybridize to reform the parent double stranded molecules, some hybridize with each other to form a heteroduplex with one strand originating from each parent. Note the two polymorphic locations, T~G and A~C in the heteroduplex whereas the first parent has T-A and A-T and the second parent has C-G and G-C respectively. T~G and A~C base pairs do NOT hydrogen bond to each other hence mismatches are present.

The present invention then resolves the mismatches by a series of reactions, which results in the first mismatched base pair becoming instead T-A or C-G. These are perfect matches. The second mismatch is likewise converted to either A-T or G-C. The reactions operate on each mismatch **INDEPENDENTLY** which can result in resolving one mismatch to resemble one parent and another mismatch to resemble a different parent. The end result is four different combinations, the two parents and two sequence variants. Each variant molecule may be separated and analyzed for whatever biological activity the polynucleotide may have.

In practice, two different versions of a gene may have several points of mismatch. One can obtain a large number of different polynucleotide sequences, theoretically 2^n different molecules or $2^n - 2$ different variants where n is the number of mismatches. Using three or more different versions of the same gene results in a considerably greater number of sequence variants in the resulting population.

The present invention performs the reaction resolving the mismatches using an *in vitro* system with an artificial combination of enzymes that have the activities of an endonuclease which is directed to and nicks or cleaves at the mismatched nucleotides, an exonuclease which removes one or more nucleotides including mismatched ones, and a polymerase to add complementary nucleotides to the strand treated with the exonuclease so that the molecule will no longer have a mismatch at that site. An enzyme having ligase activity is also preferred to

seal the nick. Because some enzymes have more than one activity, one need not have a separate enzyme for each activity. The endonuclease used in the invention is different from many conventional endonucleases because it recognizes and cleaves specifically at the mismatched nucleotide of a heteroduplex. The claimed preferred combination of enzymes is CEL I, T4 DNA polymerase, and T4 DNA ligase.

The population of sequence variants made may then be separated into individual variants, which may be separately analyzed for whatever desired biological properties one chooses. Alternatively, the population may be simultaneously screened for a desired biological property. In one embodiment of the invention, the sequence variants, with or without the parent sequences, may be used as starting polynucleotides for another round of gene shuffling according to the present invention. The resulting population of sequence variants may be even larger allowing one to choose among a greater selection of molecules.

The specification provides examples of different types of sequence variants that can be made. Model systems such as Example 3 show variant sequences with differing restriction sites. Examples 4 and 5 show making variant sequence molecules of green fluorescent protein, some affecting the fluorescent properties. Example 10 shows making variant sequences that affect the infectivity of a virus. Example 11's variant sequences are selected for affecting enzyme activity.

By using full-length polynucleotide strands to form the heteroduplex, one can reasonably expect to obtain full-length polynucleotide products. This provides a distinct advantage over previous gene shuffling techniques. Also by using a defined system with as few enzymes as practical, one minimizes side reactions and unwanted results.

(6) Issues

The primary issue before the honorable Board of Patent Appeals and Interferences is the interpretation of certain claim language. The issues are whether claim language is adequately supported by the specification within the meaning of 35 USC 112, first paragraph, and whether the same claim language is definite within the meaning of 35 USC 112, second

paragraph. The interpretation of the same claim language is also the basis for an interpretation leading to the following rejections.

Another primary issue is whether or not Claims 67, 69-73, 85, 87-90 are properly rejected under 35 USC 102(e) as being anticipated by Vind.

Building upon that issue is whether or not claim 68 is unpatentable under 35 USC 103(a) over Vind.

Likewise, there is an issue of whether or not claims 75-77 and 80 are unpatentable under 35 USC 103(a) over Vind in view of Arnold et al.

Likewise, there is an issue of whether or not claims 78 and 79 are unpatentable under 35 USC 103(a) over Vind in view of Birkenkamp et al.

Likewise, there is an issue of whether or not claims 66-74, 81-82, 85, and 87-90 are unpatentable under 35 USC 103(a) over Vind in view of Oleykowski et al.

Among the sub-issues are the following:

Are the terms, "...a defined composition ... consist essentially of..." is equivalent to "comprising"?

Are whole cell extracts the same as defined compositions with enzymes that consist essentially of a given list?

Does the prior art provide any suggestion or motivation to use a mismatch recognizing and mismatch directed endonuclease for an unrelated purpose in an unrelated composition?

Does the prior art using whole cell extract adequately suggest using a defined composition, particularly one where specific enzymes are from different types of cells?

Are the advantages achieved by the claimed method using the claimed defined composition recognized by the prior art?

Is it proper to combine components from different references when the references are performing unrelated methods?

(7) Grouping of Claims

None of claims 66-72, 78-83, 85, and 87-90 should be grouped together because many are separately patentable. The Final Rejection indicated different claims were rejected over different references and combinations of references. On its face, at least some claims may be separately patentable due to differences in the properness of the current rejections.

Only claims 67 and 78-80, 85 stand or fall together in one group and claims 70 and 72 stand together in another group. All other claims stand alone.

Specifically, claim 66 recites specific enzymes not recited in claim 67 which provide a separate reason for patentability. Indeed, the rejection involving claim 66 is based on additional references from claim 67.

Claim 68 is separately patentable over claim 67 by requiring an order of the steps for adding various enzymes. This point is critical with respect to certain alleged prior art references where all of the enzymes are added simultaneously *in vivo* or *in vitro*.

Claim 69 is separately patentable because it recites concurrent addition of the enzymes whereas the cited prior art adds the substrate to a whole cell.

Claims 70 (with 72) and 71 are separately patentable from their separate parent claims 68 and 69 by the addition of a ligase, an enzyme separate from the possible enzymes in the independent claims that have dual activities.

Claims 81-83 are separately patentable over the independent claim and over each other in the use of specific enzymes. More specifically the use of CEL I and endonuclease VII are unobvious choices of mismatch directed endonucleases because CEL I was previously thought to be involved in seed formation, an unrelated property, and endonuclease VII is well known to be difficult to work with and suffers from a lack of reproducibility.

Claim 87 is separately patentable by requiring at least two sequences variants to be produced. This claim distinguishes the invention from certain cited prior art, which disclose a mutation repair technique, which involves only one variant.

Claim 88 is separately patentable by reciting the screening or selecting of a variant sequence which distinguishes the invention from certain prior art which produces or repairs a mutation without bothering to determine the mutant sequence's functional properties.

Claim 89 is separately patentable because the sequence variant has a different functional property, thereby establishing the variant as unobvious over the parent sequences.

Claim 90 is separately patentable because by having so many mismatches and variants, it is patentably distinct from any technique handling single mutations.

Certain claims may be grouped with other claims for purposes of discussion in this appeal for various reasons with respect to various rejections. No admission of a lack of patentable difference between each other should be inferred except as grouped together above. In the argument below, appellants present arguments and discuss evidence, some of which pertain only to certain claims and not others.

(8) Argument

The rejection of claims 66-72, 78-83, 85 and 87-90 under 35 USC 112, first paragraph is based on the contention that certain language is not described in the specification and is “new matter”. The disputed language is “...defined composition containing enzymes wherein the enzymes consist essentially of...” It is found in both independent claims 66 and 67. The other claims are dependant on these two claims. The examiner’s position is that the exact phrase never appears in the specification. This is true, but as previously recognized by the examiner an exact recitation of the language is not required citing *In re Wright*, 9 USPQ2d 1649, 1650 (Fed. Cir. 1989). Numerous other examples in the case law repeat this point.

The examiner elaborates by contending that the term “defined” is not sufficiently described. He recites the example of a cell extract being undefined but questions what level of purity is needed before the purified ingredient becomes “defined” and asserts the specification does not support the meaning. This rejection is respectfully traversed.

The purpose of this language is to describe using a composition based on reagents rather than a whole cell extract containing thousands of enzymes.

Reading the entire phrase in the claim, the word “defined” becomes clearer. The claims recite, “...defined composition containing enzymes wherein the enzymes consist essentially of...” It is the enzymes that define the composition. Of course the composition may contain water but from the phrase, the “defined” nature clearly refers to the enzymes present.

Throughout the specification and in the examples, the enzymes used were highly purified. Many were purchased reagents from standard manufacturers. In an absolute sense, almost nothing is completely pure but those skilled in the art understand and accept that reagent-grade materials are considered “defined” for practical considerations. This is particularly true for enzymes, some of which have never been crystallized and even so, those skilled in the art routinely add protease inhibitors (note the PMSF used in the specification) and nuclease inhibitors to reduce the effects of contamination. Those skilled in the art reading the specification understand that it is the recited enzyme activities which are performing the claimed method and that a “defined composition” would exclude all (in a practical, not absolute sense) other relevant enzymes that might interfere.

The examiner has not contested that individual enzyme activities are defined in the specification such as on page 11, last paragraph, 12, first paragraph, page 21, first paragraph, page 22, last paragraph, etc.

The use of defined enzymes becomes even more apparent when the language is used in the context of certain claims. Independent Claim 66 recites “...a defined composition containing enzymes wherein the enzymes consist essentially of an effective amount of CEL I, T4 DNA polymerase, and T4 DNA ligase...” The defined enzymes are the three specific enzymes recited, namely CEL I, T4 DNA polymerase, and T4 DNA ligase. Various dependant claims are also specific about the enzymes and their sources.

Those skilled in the art reading the specification would see that reagents are preferred throughout and understand that a “defined composition” would be made of purified components. Throughout the specification, only purified enzyme components are used in the claimed method. Every example and every description of the claimed method use purified enzymes. In the paragraph bridging pages 61 and 62 there is a discussion that changing the relative concentrations of certain enzymes results in changed results or even prevented any results from occurring at all. Later on page 62, lines 17+ relative concentrations were optimized. Example 6 is entirely devoted to studying the interactions of the enzymes and defining preferred compositions. This indicates that even the relative concentrations of the enzymes should be controllable for certain results. Skilled artisans reading these passages will

recognize the inherent support for “defined compositions” and understand the term’s purpose in the claims.

Even the examiner has an understanding of the meaning of “defined composition” from reading the specification because he states in the final rejection on page 3, lines 17-18 “...purified set of components, all of which could be identified, which is closer to what the ordinary practitioner would understand “defined” to mean.” Accordingly, this rejection should be reversed.

Claims 66-72, 78-83, 85 and 87-90 were rejected under 35 USC 112, second paragraph as allegedly indefinite over the same terms as above. The examiner contends that “defined composition” is vague and indefinite as it may be less than homogenously purified proteins. The language is found in both independent claims 66 and 67. The other claims are dependant of these two claims. This rejection is respectfully traversed.

To under stand the term “defined composition”, it should be viewed in context. The claims state “...a defined composition containing enzymes wherein the enzymes consist essentially of an effective amount of...” It is the types of enzymes present which are defined. The enzymes or activities recited need to be present and by implication are the ones functioning in the claimed method. The arguments above and passages in the specification supporting the use of the term “defined composition” also clarify the term’s meaning and are equally applicable to this rejection as well.

To highlight a less than absolutely pure enzyme, the examiner points to the CEL I preparation from Example 1. From this the examiner concludes the term “defined” is indefinite.

This is contradicted by the art recognized meaning for this enzyme. Oleykowski et al. was cited by the examiner as teaching the same enzyme. Appellants use a purification protocol in Example 1 that is a multiple step purification procedure, including an affinity purification (well known for great enrichment in one step). Appellants’ purification includes all of the steps published by Oleykowski et al. (and further steps also). Since the prior art recognizes that published procedure as producing a defined enzyme, then appellants’ procedure which incorporates that published procedure should be considered to produce at least as defined of an

enzyme. Therefore, the alleged basis for concluding that this claimed language is vague and indefinite would necessarily indicate that a peer-reviewed journal article is also vague, indefinite and does not define what it supposedly does. Appellants assert Nucleic Acids Research is generally a well-respected journal that is unlikely to publish sloppy work.

The examiner further states in the final rejection, on page 4, lines 18-20, “The specification does not teach or provide any situation where the entire composition is known and the leve [level] of each component precisely defined...” This assertion is disputed.

Example 9 (page 74, lines 25+) states:

“Reactions were set up using twenty-one nanograms of the circular double-stranded heteroduplexed GFP plasmid substrate in ten microliters containing 1X NEB ligase buffer, 0.5 mM each dNTP, 0.2 units T4 DNA ligase (Gibco/BRL), 1 unit of T4 DNA polymerase, and either 1.0 microliter of CEL I purified from celery (fraction 5, described in Example 1), or 0.3 microliters of CEL I purified from a cloned source.”

This is an example of what “defined” is as described by the specification. The level of precision may not be sufficiently great to state that 1.00000000000000 microliters was used but the term defined is clear from the specification. Therefore, the claims are neither vague nor indefinite and this rejection should be reversed.

Regarding the rejections over prior art, Any possible combination of all of these teachings still does not suggest the claimed method of making many sequence variants by forming and resolving a heteroduplex with a mismatch directed endonuclease, exonuclease, polymerase and ligase activities. Any possible combination of teachings would not suggest obtaining the unexpected result from combining such activities to a heteroduplex substrate.

It is well established that every aspect of the claimed invention must be shown or suggested by the combination of references. This was not done for the present rejection.

In the absence of any suitable showing of any of these requirements for a proper rejection under 35 USC 102 or 103, the claims must be deemed patentably unobvious.

Claims 67, 69-73, 85 and 87-90 were rejected under 35 USC 102(e) as being anticipated by Vind. This contains a typographical error and should read 69-72 because claim

73 has been canceled. Vind prepares sequence variants by mixing two different double stranded polynucleotides with a whole cell extract containing DNA repair enzymes, heat denaturing the polynucleotides, annealing to form a heteroduplex and allowing the enzymes in the whole cell extract to repair the mismatches thereby producing the parents and sequence variants. The examiner has interpreted the claimed phrase “defined composition” to be the cell extract of Vind. See page 5, line 11-13. The examiner has also interpreted ““consisting essentially of” will be construed as equivalent to “comprising”.” See page 5, line 16-17. For several reasons, this rejection is respectfully traversed regardless of this misguided interpretation.

First, it is unreasonable to interpret the Vind cell extract as a “defined composition”. Vind uses homogenized thermostable bacteria as their cell extract which is taught to contain, or rather is presumed to contain the entire DNA repair system. Such a cell extract would have thousands of enzymes in unknown concentrations. No attempt at purification is ever made. This is the opposite of “defined”.

Second, an extract with thousands of different enzymes cannot be considered to anticipate a composition recited in the claims as “...wherein the enzymes consist essentially of an effective amount of...” listing the enzyme activities. The differences between “comprising” and “consisting essentially of” as transitional phrases have been thoroughly described in the case law, a summary of which is found in MPEP 2111.03. The term “consisting essentially of” encompasses less than “comprising” and in the present situation must mean something less than an undefined mixture of thousands recited in Vind. Furthermore, appellants recite a short list of only 3 or 4 specified enzymes/activities. This language alone would exclude the entire DNA repair system which would interact on the same substrates as in appellant’s process and which Vind considers critical to the functioning of his invention. Note column 5, lines 27+ for the definition of “mismatch repair system” and column 8, lines 17-27.

Third, even accepting the examiner’s misinterpretation, Vind does not anticipate the present claims. The “mismatch recognizing and mismatch directed endonuclease that cleaves at the mismatched nucleotides” (e.g. CEL I) is not taught by Vind nor is it reasonable to assume it to be inherently present. This enzyme is not part of the normal DNA repair system

and therefore not suggested by Vind. The normal bacterial DNA repair system may recognize mismatches but the endonuclease(s) cleave somewhere upstream or downstream from the mismatch. Therefore, Vind's DNA repair system's endonuclease is neither "mismatch directed", nor does it "cleave at the mismatched nucleotides".

Furthermore, in nature, this enzyme is believed to be involved in the distribution of nutrients during production of seeds in plants. Vind uses bacterial extracts. There is no reason to expect a plant-specific enzyme to be present in bacteria. Therefore, it is improper to assume its presence in Vind.

Fourth, regardless of what compositions are used, the method steps differ in the claimed method. The claims recite forming the heteroduplex first followed by adding the enzymes. Vind adds enzymes to plural double stranded nucleic acids followed by forming the heteroduplex. Because the order differs, technically, the claims cannot be considered anticipated under 35 USC 102.

As for the logical question of whether the order of steps matters or would be obvious under 35 USC 103, appellants perform their order of steps because the heat used to melt and anneal the nucleic acid strands to form the heteroduplex would inactivate the enzymes. By contrast, Vind mixes enzymes with double stranded nucleic acid before melting and annealing the nucleic acid strands. Because Vind specifically requires thermostable enzymes (column 31, line 53), he is not concerned with heat inactivation. From Vind, there is no motivation to change the order of steps, particularly since Vind specifically chose thermostable enzymes specifically adapted to their order of steps. Accordingly, a rejection under 35 USC 103 would not apply either.

Fifth, the claimed method recites contacting the recited enzyme activities with the heteroduplex substrate. This at least implies that the enzyme activities actually do react on the heteroduplex substrate as indicated in the specification to obtain the recited linear sequence variants. Vind teaches contacting DNA repair enzymes with their heteroduplex and presumably they react as indicated in Vind. The enzymes and actions differ and therefore the method is different even if the final product is the same. Therefore the method cannot be considered anticipated.

Sixth, the combination of enzymes used by Vind are naturally occurring, naturally working together as they are called a DNA repair system and are obtained by a single cellular extract. By contrast, the enzymes used in the present invention are not believed to occur together in nature, nor do they act together for a particular purpose. The claimed enzyme composition is formed as a totally artificial in-vitro system working together to form a novel and artificial series of reactions. For example, in all of the specification Examples, the enzymes are obtained from two or more different species. Therefore, the claimed method using such combinations is clearly different from and not anticipated by Vind.

Seventh, Claim 71 requires addition of ligase in a separate step and at a separate time from the addition of the other enzymes. Vind adds the composition with all of their enzymes at one time. Therefore, Vind cannot anticipate this claim.

Eighth, Claims 87 and 90 recite recovering plural different sequence variants and using a heteroduplex having at least three mismatches. While Vind hopes to generate a library of many variants, Vind shows recovery of only one, not plural different sequence variants. The final screening assay in Example 7 is qualitative and apparently does not distinguish between two similar but different sequence variants. Likewise, where is it shown that the Vind heteroduplex contains at least three mismatches? While Vind may hope to discover a library of many sequence variants and one might imagine using many different types of heteroduplexes, Vind does not actually show that such can be done. Therefore, application of section 102 to these claims is technically not appropriate.

The examiner has argued that the claims encompass plural enzymes, which presumably would be met by the DNA repair (Mut) system of Vind. The claims recite “a mismatch recognizing and mismatch directed endonuclease”. The term endonuclease is singular. A singular enzyme is not anticipated by a series of plural enzymes allegedly performing the same function. Also, even using the entire system in Vind, Vind still lacks cleavage at the mismatched nucleotides. DNA repair generally cleaves upstream or downstream of the mismatch, frequently very far upstream and downstream. This is not exactly “at the mismatched nucleotides” as claimed.

For all of these reasons, the rejection should be reversed.

Claim 68 was rejected under 35 USC 103(a) as being unpatentable over Vind. Applying Vind as above, the examiner contends that adding the ingredients in any particular order is *prima facie* obvious in the absence of any unexpected results regarding the order of addition.

As a general principle appellants agree, but this is not a proper characterization of the facts. Claim 68 does NOT differ from Vind by a simple different order of addition. Vind uses one composition for one addition. Vind cannot alter their composition because it is a single composition with all of the enzymes in it. Vind cannot alter the order of addition because there is only one composition to add. There is no permitting (or motivation) for adding a separate composition at a later time. Claim 68 recites adding three different compositions at three different times. By having plural compositions to add, a sequential order is possible and indeed is being claimed. Therefore the rejection should be reversed.

As a second matter, the order of addition has been shown to be important to some but not all of the embodiments of the invention. The two embodiments on page 46-47 of the specification recite allowing one enzyme to work on the substrate followed by a later addition and another enzyme. Also, it should be noted that in Examples 2 and 6 where different amounts of enzymes are used with differing results. Likewise, one may infer that having one enzyme added first and exposing it to the heteroduplex for a longer period of time is similarly effective to using a larger amount of enzyme. As such, one may expect a different order of addition to have different effects. Therefore, for this reason also, the rejection should be reversed.

Claims 75-77 and 80 were rejected under 35 USC 103(a) as being unpatentable over Vind in view of Arnold et al. Claims 75-77 have been canceled and thus the rejection should read only claim 80. In addition to the application of Vind as above, Arnold et al. is cited to show another DNA repair system used for mismatch correction using E. coli extracts which presumably contain E. coli Pol 1. The examiner argues that to substitute equivalents between the two systems would be obvious. This rejection is respectfully traversed.

Adding Arnold et al. does nothing to overcome the deficiencies of Vind referred to in responding to the rejections over Vind alone. This is especially so with respect to the “mismatch recognizing and mismatch directed endonuclease that cleaves at the mismatched nucleotides” (e.g. CEL I) which is not hinted at in Arnold et al. Claim 80 recites that the enzyme is E. coli Pol 1. This enzyme is not present in anything other than a whole cell or cell extract and therefore is not available for adding to the Vind DNA repair system. While Arnold et al. may contain the same enzyme in the cells used in their DNA repair system, one would not be motivated to simply pull it out and add it to the Vind DNA repair system because E. coli Pol 1 would not operate in the Vind method. Vind specifically recites using thermostable enzymes. The ordinary skilled artisan knows that E. coli Pol 1 is NOT thermostable. Vind subjects his enzymes to temperatures sufficiently high to melt and anneal the polynucleotide strands into a heteroduplex. This temperature would inactivate E. coli Pol 1. Note the examples expose the enzymes (cell extract) to PCR conditions of 95° C and 72° C, clearly too hot for E. coli Pol 1. Therefore, one lacks motivation to combine the references in the manner suggested by the examiner because it would be expected to be inoperable.

The examiner has argued that Vind has a preferred embodiment of using high temperature enzymes but this does not prevent the use of alternative embodiments. However, this mischaracterizes Vind. All embodiments of Vind require high temperature enzymes because all embodiments in Vind subject the enzymes to temperatures sufficiently high to melt and anneal double stranded polynucleotides. The examiner has pointed to column 5, line 56, where a human enzyme is mentioned. However, reading the entire sentence it is clear that it is the homologue of that human enzyme in the bacterium *T. aquaticus* being discussed.

It is established that when a reference teaches away from what the Examiner contends is obvious, the rejection is improper. In re Grasselli et al., (CAFC 1983) 218 USPQ 769. In the present situation, the Vind reference requires the use of thermostable enzymes in a high temperature environment. The use of enzymes that would be denatured would not be obvious, such as those in Arnold et al.

Accordingly, this rejection should be reversed.

Claims 78 and 79 were rejected under 35 USC 103(a) over Vind taken in view of Birkenkamp et al. In addition to Vind being applied as above, Birkenkamp et al. is cited to show using the T4 mismatch correction system. The examiner contends it obvious to use it in the mismatch repair system of Vind as a substituted equivalent part of a DNA repair system. This rejection is respectfully traversed.

Birkenkamp et al. does not compensate for the basic deficiencies in Vind as set forth in the rejections over Vind alone. Birkenkamp et al. performs DNA repair yielding one or both parent strands. There is no generation of sequence variants in Birkenkamp et al. Therefore, one must use Birkenkamp et al.'s DNA repair system to modify Vind and not vice versa.

As noted before regarding Arnold et al., adding the T4 enzyme(s) to the Vind system would not be motivated because it would yield an inoperable system. T4 enzymes are generally not thermostable and would be inactivated at the temperatures used by Vind. Substituting equivalents is typically obvious but not when the substitution would be expected to fail and would be taught away. When a reference teaches away from what the Examiner contends is obvious, the rejection is improper. In re Grasseli et al., (CAFC 1983) 218 USPQ 769.

Again, like Vind, Birkenkamp et al lacks the "mismatch recognizing and mismatch directed endonuclease that cleaves at the mismatched nucleotides" (e.g. CEL I) and its use in generating sequence variants.

As this combination of references does not render the instant claims obvious, this rejection should be reversed.

Claims 66-74, 81-82, 85 and 87-90 were rejected under 35 USC 103(a) as being unpatentable over Vind in view of Oleykowski et al. Claims 73 and 74 had been canceled and therefore the rejection should read 66-72, 81-82, 85 and 87-90. The examiner asserts that Vind discloses the general method except for the use of CEL I. Oleykowski et al is cited as teaching CEL I and its use for mismatch "correction". The examiner further contends that it would be obvious to substitute CEL I into the cell extract DNA repair system of Vind. This rejection is respectfully traversed.

Oleykowski et al. does not compensate for the basic deficiencies in Vind as set forth in the rejections over Vind alone. Oleykowski et al. performs DNA mutation detection by nicking at the site of a mismatch, separating the broken strands and characterizing the site of the cut by measurement of the fragments. There is no generation of sequence variants in Oleykowski et al. Oleykowski et al does not even desire to generate variant sequences. Even more telling, Oleykowski et al does NOT use CEL I in any way related to DNA repair. In order for one to be motivated to add the CEL I enzyme to the Vind DNA repair system, there must be some reason to believe the enzyme would function in the DNA repair system. The only taught use for CEL I is that it fragments DNA, the opposite from repairing DNA. Therefore, one must use Oleykowski et al.'s DNA mutation detection system to modify Vind and not *vice versa*.

The examiner has contended one would have two motivations to use CEL I in the method of Vind. (page 15, last paragraph of the final rejection) First, CEL I operates differently from T4 endonuclease VII with certain advantages. Such an assertion provides no motivation. Vind does not mention using either T4 endonuclease VII or CEL I. Therefore substituting one for the other still does not involve the Vind method. Second, the examiner asserts CEL I is superior for mutation detection. That may be true also but that has nothing to do with the Vind method. In both motivations and both references, CEL I is not involved in generating a sequence variant (or anything close to it). Therefore, no connection has been made to the method of Vind.

Pulling a single feature from reference to combine to another is a classic example of using hindsight. There is nothing in Oleykowski et al to suggest the preparation of a new polynucleotide with a sequence variation. Likewise, Vind is completely devoid of any suggestion to add an enzyme other than one used in the DNA repair system. As noted above, no motivation, connection or common purpose has been suggested to make such a combination.

Further, as noted above regarding Arnold et al., there is no motivation to add CEL I enzyme to the Vind system because it would be inoperable in the system. CEL I is not highly thermostable and would be inactivated at the temperatures used by Vind. Substituting equivalents is typically obvious but not when the substitution would be expected to fail.

Even if one were to attempt to use CEL I in Vind, CEL I would not function due to its thermal instability. As such, the reference teaches away from the present invention. Making an invention contrary to the teachings of the prior art is not obvious. When a reference teaches away from what the Examiner contends is obvious, the rejection is improper. In re Grasseli et al, (CAFC 1983) 218 USPQ 769.

Furthermore, it is not clear how CEL I would function in conjunction with any DNA repair system, even one operating at room temperature. Since DNA repair does not involve a mismatch directed endonuclease such as CEL I, the effect of adding CEL I is unclear. Therefore, such a combination would not be suggested to one of ordinary skill in the art and this rejection should be reversed.

(10) Legal Basis for Certain Arguments

As repeated by a small library of case law, use of the specification for providing motivation and expectation of successful results is "hindsight" and not permitted. Throughout the rejections, the examiner is consistent by not showing such suggestions in the references themselves.

Support for showing an invention to be unobvious due to references' contrary teachings and teaching away from the invention is found in In re Grasseli et al, (CAFC 1983) 218 USPQ 769.

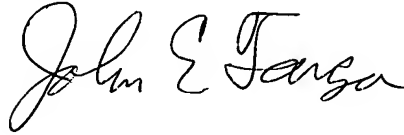
CONCLUSION

The Examiner has not established a prima facie case of obviousness. He has not even shown how the references disclose the critical recitations in the claims or how the references would be combined to disclose the claimed invention with its operable abilities. Accordingly, for these and all the other reasons given above and during prosecution at the Examiner's level, the rejection should be reversed. Appellants request the honorable Board of Appeals to do so.

To the extent necessary, appellants petition for an extension of time under 37 C.F.R.

§1.136. Please charge any shortage in fees due in connection with the filing of this paper, including extension of time fees, to the Deposit Account No. 500933, and please credit any excess fees to such Deposit Account.

Respectfully submitted,

A handwritten signature in black ink, reading "John E. Tarcza". The signature is fluid and cursive, with the first letters of each name being capitalized and prominent.

John E. Tarcza

Registration No. 33,638

Attachments: Appendix list of claims
Petition for Extension of Time

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Appendix

Claim 66. An in vitro method of making linear sequence variants from at least one heteroduplex polynucleotide where said heteroduplex has at least two non-complementary nucleotide base pairs separated by complementary nucleotide base pairs, said method comprising:

- a. preparing at least one heteroduplex polynucleotide;
- b. combining said heteroduplex polynucleotide with a defined composition containing enzymes wherein the enzymes consist essentially of an effective amount of CEL I, T4 DNA polymerase, and T4 DNA ligase;
- c. allowing sufficient time for the percentage of complementarity to increase, wherein one or more sequence variants are made thereby increasing the diversity in a population of polynucleotides; and
- d. separating and recovering at least one sequence variant having a sequence different from either polynucleotide strand in said heteroduplex.

Claim 67. An in vitro method of making linear sequence variants from at least one heteroduplex polynucleotide wherein said heteroduplex has at least two non-complementary nucleotide base pairs separated by complementary nucleotide base pairs, said method comprising:

- a. preparing at least one heteroduplex polynucleotide;
- b. combining said heteroduplex polynucleotide with a defined composition containing enzymes wherein the enzymes consist essentially of an effective amount of an enzyme or enzymes with exonuclease activity, polymerase activity and a mismatch recognizing and mismatch directed endonuclease that cleaves at the mismatched nucleotides;
- c. allowing sufficient time for the percentage of complementarity to increase, wherein at least one or more sequence variants are made thereby increasing diversity in a population of polynucleotides; and

d. separating and recovering at least one sequence variant having a sequence different from either polynucleotide in the heteroduplex.

Claim 68. The method of claim 67 wherein said endonuclease is added first, the enzyme or enzymes having 3' to 5' exonuclease activity is added second, and the enzyme or enzymes having polymerase activity is added third.

Claim 69. The method of claim 67 wherein said enzymes having exonuclease activity, polymerase activity, and endonuclease are added concurrently.

Claim 70. The method of claim 67 in step (b) further comprising ligase activity.

Claim 71. The method of claim 69 further comprising a step of, (d) adding a ligase.

Claim 72. The method of claim 70 wherein said ligase is T4 DNA ligase, E. coli DNA ligase, or Taq DNA ligase.

Claim 78. The method of claim 67 wherein said agent with polymerase activity is T4 DNA polymerase.

Claim 79. The method of claim 67 wherein said enzyme with both polymerase activity and 3' to 5' exonuclease activity is T4 DNA polymerase, T7 DNA polymerase, E. coli Pol 1, or Pfu DNA polymerase.

Claim 80. The method of claim 67 wherein said enzyme with both polymerase activity and 5' to 3' exonuclease activity is E. coli Pol 1.

Claim 81. The method of claim 67 wherein said effective amount of said endonuclease, and exonuclease activity/polymerase activity and ligase activity are provided by CEL I, T4 DNA polymerase, and T4 DNA ligase.

Claim 82. The method of claim 67 wherein said effective amount of said endonuclease, and exonuclease activity/polymerase activity and ligase activity are provided by CEL I, T7 DNA polymerase, and T4 DNA ligase.

Claim 83. The method of claim 67 wherein an effective amount of said endonuclease, and exonuclease activity/polymerase activity and ligase activity are provided by T4 endonuclease VII, T4 DNA polymerase, and T4 DNA ligase.

Claim 85. The method of claim 67 wherein complementarity is complete yielding a homoduplex polynucleotide.

Claim 87. The method of claim 67 wherein at least 2 different polynucleotide sequence variants are formed and recovered.

Claim 88. The method of claim 67 further comprising screening or selecting a population of sequence variants for a desired functional property.

Claim 89. The method of claim 88 further comprising selecting a sequence variant that has a different desired function property from any parent polynucleotide.

Claim 90. The method of claim 67 wherein said at least one heteroduplex polynucleotide has at least three non-complementary nucleotide base pairs separated by complementary nucleotide base pairs and at least 4 different sequence variants made.